REMARKS

Any fees that may be due in connection with filing this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-19, 22-24, 26-38, 52-61, 63-67, 69-71, 73-78 and 85 are pending. Claims 70, 71, 73-78 and 86 are cancelled herein. Claim 1 is amended to replace the density range of "1.060-1.067" with a range of "1.060-1.065" g/ml. Basis for amended Claim 1 may be found in the specification, *e.g.*, at page 18, lines 10-14. No new matter has been added.

An executed DECLARATION under 37 C.F.R. §1.132 of Dahm is attached hereto.

Amendments and responses filed responsive to all previous Office Actions that issued in connection with the above-captioned application are incorporated by reference herein.

THE REJECTION OF CLAIMS 1-19, 22-24, 26-38, 52-61, 63-67, 69-71, 73-78 AND 85 UNDER 35 U.S.C. § 103(a)

A. REJECTION OF CLAIMS 1, 2, 4, 7-11, 14-19, 22-24, 26-28, 34-38, 52-56, 60, 61, 63, 64, 67, 69-71, 73, 75-78 and 85 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND FURTHER IN VIEW OF BOSSLET *ET AL.*

Claims 1, 2, 4, 7-11, 14-19, 22-24, 26-28, 34-38, 52-56, 60, 61, 63, 64, 67 and 69-71, 73, 75-78 and 85 are rejected under 35 U.S.C. §103(a) over Cech *et al.* (U.S. Patent No. 6,166,178) in view of Van Vlasselaer (U.S. Patent No. 5,648,223) and further in view of Bosslet *et al.* (*Br. J. Cancer 44*:356-362 (1981)). Cech *et al.* allegedly provides a method of quantitating tumor cells in a body fluid by concentrating tumor cells in a sample of body fluid, amplifying mRNA coding for the catalytic subunit of telomerase (hTRT) and quantitatively determining the amount of amplified nucleic acid. Van Vlasselaer allegedly teaches methods for enriching tumor cells by centrifugation where the density of the cell separation medium is adjusted to the density of the cell type. Bosslet *et al.* allegedly teaches a centrifugation-based method of separating tumor cells

using density gradients and is further alleged to teach that in linear Percoll density gradients, tumor cells are found in bands ranging from 1.051 to 1.068 g/ml, whereas non-tumor cells are found in bands ranging from 1.075 to 1.082 g/ml.

The Examiner alleges that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the instant application was filed to have modified the method of Cech *et al.*, which allegedly teaches a method of quantitating tumor cells in a body fluid by quantitating the amount of amplified mRNA encoding the catalytic subunit of telomerase in tumor cells that are concentrated from the body fluid, with Van Vlasselaer, which allegedly teaches centrifugation-based methods for enriching tumor cells prior to analysis, and further using a cell separation medium density of 1.060-1.067 for separating tumor cells from non-tumor cells as allegedly taught by Bosslet *et al.*, to arrive at the instantly claimed subject matter.

The Office Action acknowledges that the instant application discloses 1.060-1.067 g/ml as an optimal density range for separation of tumor cells from telomerase-positive non-tumor cells in a body fluid. The Examiner infers that the critical limit of the range is the upper limit of 1.067 g/ml because normal cells are found in bands nearing the upper limit. The Examiner then concludes that, barring a showing of unexpected results with an upper density limit of 1.067 g/ml as instantly claimed compared to an upper density limit of 1.068 g/ml as allegedly taught by Bosslet *et al.*, the claims "share significant overlap" with the teachings of Bosslet *et al.* and the *prima facie* case of obviousness is not overcome.

Responsive to Applicant's arguments that neither of the cited references, singly or in combination, teaches or suggests the instant methods for quantification of tumor cells in a body fluid, the Examiner rebuts that Cech *et al.* teaches quantifying the amount of amplified nucleic acid. The Examiner states that Applicant's argument that Cech *et al.* does not teach or suggest correlating the amount of amplified mRNA to the number of tumor cells in the sample is

unpersuasive because of Applicant's statement in the response filed September 8, 2003, which stated that correlating the amount of amplified mRNA encoding a gene and the number of cells expressing that gene was routine to those of ordinary skill in the art as of the earliest priority date of the application.

Reconsideration and withdrawal of this rejection is respectfully requested in view of the amendments herein and the following remarks. It is respectfully submitted that this rejection has been rendered moot with respect to Claims 70, 71, 73-78 and 86, which are cancelled herein.

Summary of Arguments

1) None of the cited references, singly or in any combination, teaches or suggests a method for quantification of tumor cells

As discussed previously, the teachings of Cech *et al.* and Van Vlasselaer, singly or in combination, does not result in the instantly claimed methods because neither of the cited references, singly or in combination, teaches or suggests any method for quantifying circulating tumor cells in a body fluid.

The Examiner alleges that Cech *et al.* does teach quantification of tumor cells because Cech *et al.* teaches quantifying the amount of amplified catalytic subunit of telomerase and, as Applicant allegedly admits in the response filed September 8, 2003, the step of correlating the amount of amplified mRNA with the number of tumor cells was routine and well-known to those of ordinary skill in the art at the time the instant application was filed.

In response, Applicant respectfully submits that regardless of whether or not such correlation is routine, there is no teaching or suggestion in Cech *et al.* to perform such a correlation and to measure the number of tumor cells in a biological sample. The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ 1783 (Fed. Cir. 1992). Unlike the instant application, Cech *et al.* provides no teaching or suggestion that it would be desirable to quantitate

tumor cells, much less how one of ordinary skill in the art would measure the quantity of tumor cells.

Bosslet *et al.*, which also does not teach or suggest any method for quantitation of tumor cells, does not cure the deficiencies of Cech *et al.* and Van Vlasselaer. Therefore, the combination of the cited references does not result in a method for the quantification of tumor cells in a body fluid as instantly claimed.

2) None of the cited references, singly or in any combination, teaches or suggests concentration of tumor cells from a body fluid using a range of cell separation medium density (1.060-1.065 g/ml) as instantly claimed. Further, as shown by the attached DECLARATION under 37 C.F.R. §1.132 of Dahm, the density range of 1.060 - 1.065 g/ml that is an element of the pending claims provides results that are not taught or suggested by the cited references, singly or in any combination.

The claims as amended herein specify that the tumor cells are concentrated from a body fluid using a cell separation medium in the density range of 1.060-1.065 g/ml. Neither Cech *et al.* nor Van Vlasselaer teaches or suggests a density range for enrichment of tumor cells from a biological sample such as a body fluid. Bosslet *et al.* teaches separation of tumor cells using linear Percoll density gradients ranging from 1.051 to 1.068 g/ml to concentrate the rumor cells, while the non-tumor cells are found at higher densities ranging from 1.075 to 1.082 g/ml. Neither Bosslet *et al.*, nor any of the cited references, singly or in any combination, teaches or suggests a narrow density range as instantly claimed for the concentration of tumor cells from a body fluid. As discussed below, the density range of 1.060-1.065 g/ml as instantly claimed permits quantitation of tumor cells with an accuracy that is not taught or suggested by the cited references, singly or in any combination.

As demonstrated in the executed DECLARATION under 37 C.F.R. §1.132 of Dahm provided herein, using a density below 1.060 g/ml for concentrating tumor cells from a body fluid leads to significant losses of tumor cells from the tumor cell fraction. The DECLARATION further demonstrates that densities greater than about 1.065 g/ml result in contamination of the tumor cell fraction

with telomerase positive non-tumor cells. Thus, in a method for the quantification of tumor cells from a body fluid by correlating amplified catalytic subunit of telomerase with the number of tumor cells as instantly claimed, any density below 1.060 g/ml would impact the accuracy of quantitation by causing significant tumor cell loss, while any density above about 1.065 g/ml would impact the accuracy of quantitation by amplifying the catalytic subunit of telomerase from the contaminating telomerase positive non-tumor cells.

Therefore, the values of the density range taught by Bosslet *et al.* for the enrichment of tumor cells that are below 1.060 g/ml (1.051-1.059 g/ml), would lead to unacceptable losses of tumor cells that would prevent accurate quantitation of the tumor cells in a biological sample. Further, the density range at which contaminating non-tumor cells may be found in the tumor cell fraction and impact accurate quantitation of the tumor cells is much lower (1.065-1.070 g/ml) than the density range of 1.075-1.082 g/ml taught by Bosslet *et al.*

3) Conclusion

None of the cited references, singly or in any combination, teaches or suggests (1) a method for the quantification of tumor cells in a body fluid; and (2) using a cell separation medium density range of about 1.060 - 1.065 g/ml for the concentration of tumor cells from the body fluid prior to quantitation. Further, as shown by the attached DECLARATION of Dahm, the density range of 1.060-1.065 g/ml provides results for the accurate quantitation of tumor cells that are not taught or suggested by the cited references, singly or biological sample such as a body fluid as instantly claimed. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Analysis

As amended herein, the rejected claims (Claim 1 and dependents 2, 4, 7-11, 14-17, 37, 38, 52-56 and 67) are directed to a method for the quantification of tumor cells in a body fluid by (a) concentrating tumor cells in a sample of a body fluid by covering a cell separation medium with a density in the range of from 1.060-1.065 g/ml with a layer of the body fluid, centrifuging

the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid; (b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; (c) quantitatively determining the amount of amplified nucleic acid; and (d) correlating the amount of amplified nucleic acid with the number of tumor cells in the body fluid.

Thus, all the rejected claims are directed to a method for quantification of tumor cells in a body fluid that includes (i) concentration of tumor cells from the body fluid using a cell separation medium of density in the range of 1.060 - 1.065 g/ml to obtain a fraction that is enriched for tumor cells; and (ii) quantitation of tumor cells present in the enriched tumor cell fraction by correlating the amount of amplified mRNA coding for the catalytic subunit of telomerase with the number of tumor cells in the body fluid.

As discussed below, none of the cited references, singly or in any combination, teaches or suggests a method for quantification of tumor cells in a body fluid. Further, as discussed below, none of the cited references, singly or in any combination, teaches or suggests concentration of tumor cells from the body fluid prior to their quantification using a density range (1.060 - 1.065 g/ml) that optimizes the accuracy of quantification.

The teachings of the cited references and differences from the claimed subject matter

Cech *et al.*

The teachings of Cech *et al.* are of record in the response filed September 8, 2003 and in earlier responses of record in the file history of the instant application. Briefly, Cech *et al.* teaches nucleic acids encoding the catalytic subunit of telomerase and related polypeptides; amplification and quantitation of mRNA coding for the catalytic subunit of telomerase; and diagnosing telomerase-related conditions such as cancer by measuring the amount of catalytic subunit of telomerase in cells in a biological sample relative to the

normal value for those cells, where an amount that is elevated relative to the normal value is indicative of cancer.

Cech *et al.* does not teach or suggest a method of quantification of tumor cells in a body fluid in which the tumor cells are concentrated from the body fluid. At page 8 of the Office Action, the Examiner counters that Cech *et al.* does teach quantification of tumor cells because Cech *et al.* teaches quantifying the amount of amplified catalytic subunit of telomerase and, as Applicant admits in the response filed September 8, 2003, the step of correlating the amount of amplified mRNA with the number of tumor cells was routine and well-known to those of ordinary skill in the art at the time the instant application was filed.

In response, Applicant respectfully submits that regardless of whether or not such correlation is routine, there is no teaching or suggestion in Cech *et al.* to perform such a correlation and to measure the number of tumor cells in a biological sample. The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the Applicant has done. *Stratoflex Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ 1783 (Fed. Cir. 1992). As discussed previously, there is basis throughout the instant application for (1) the desirability of quantitating tumor cells, *e.g.*, as an indication of metastasis (*see* specification, for example, at page 14, lines 24-26 and at page 26, lines 3-11); and (2) how to measure the number of tumor cells after quantitating the amount of catalytic subunit of telomerase (*see* Figures and Examples throughout the specification, discussed previously, that provide detailed descriptions and demonstrations of how a correlation can established between the amount of amplified catalytic subunit of telomerase and the quantity of tumor cells). Cech *et al.* provides no teaching or suggestion that it would be desirable to quantitate tumor cells, much less how one of ordinary skill in the art would measure the quantity of tumor cells. As discussed

previously, Cech *et al.* teaches that cancer can be diagnosed in a patient by measuring the amount of catalytic subunit of telomerase in a patient's cell relative to the normal value for that cell, where an amount that is elevated relative to the normal value is indicative of cancer. There is no teaching or suggestion in Cech *et al.*, nor any of the other references cited by the Examiner, to measure the quantity of tumor cells.

There is also no teaching or suggestion in Cech *et al.* of concentrating tumor cells by centrifugation, much less using a cell separation density in a range that optimizes tumor cell quantitation by minimizing tumor cell loss while separating tumor cells from telomerase-positive non-tumor cells.

Van Vlasselaer

The teachings of Van Vlasselaer are of record in the response filed September 8, 2003 and in earlier responses of record in the file history of the instant application. As discussed in the response filed September 8, 2003, and previously, Van Vlasselaer does not cure the deficiencies in the teachings of Cech *et al.* Van Vlasselaer is directed to a method for enriching breast tumor cells from body fluids. Van Vlasselaer does not teach any diagnostic assay for the detection or quantification of tumor cells in general in a body fluid. Further, Van Vlasselaer does not teach or suggest amplification and quantitation of the mRNA for the catalytic subunit of telomerase, or any other mRNA, for the quantification of tumor cells in a body fluid. Furthermore, there is no teaching or suggestion of a concentration step as claimed in the instant methods, where concentration of the tumor cells is effected regardless of the tissue or cell type from which the circulating tumor cells originated, and there is no teaching or suggestion in Van Vlasselaer of the separation of tumor cells from telomerase-positive non tumor cells. Van Vlasselaer also does not teach or suggest a particular range of cell separation medium density such that telomerase-positive non tumor cells are separated from the enriched tumor cell fraction, while avoiding a significant loss of tumor cells.

Bosslet *et al.*

Bosslet *et al.* is directed to methods of separating metastasizing tumor cells from their host tissue or invaded tissue or body fluid, such as blood. Bosslet *et al.* teaches that such separation may be achieved using linear Percoll density gradients ranging from 1.051 to 1.068 g/ml to concentrate the rumor cells, while the non tumor cells are found at higher densities ranging from 1.075 to 1.082 g/ml (*see, e.g.*, page 357, col. 2 under "Results").

Bosslet *et al.* does not teach or suggest any method for quantitation of tumor cells, much less doing so by correlating amplified mRNA coding for the catalytic subunit of telomerase with the number of tumor cells. Further, Bosslet *et al.* does not teach or suggest a density range that enriches tumor cells while depleting telomerase-positive non tumor cells and avoiding excessive loss of tumor cells to permit accurate quantitation of the tumor cells concentrated from the body fluid. As demonstrated in the executed DECLARATION under 37 C.F.R. §1.132 of Dahm provided herein, densities of below 1.060 g/ml lead to excessive losses of tumor cells from the enriched fraction. Therefore, the lower limit of the density range taught by Bosslet *et al.*, 1.051 g/ml, would lead to unacceptable losses of tumor cells that would prevent accurate quantitation of the tumor cells in a biological sample. Further, as demonstrated by the enclosed DECLARATION of Dahm, significant numbers of non-tumor cells, some of which are telomerase positive and would therefore interfere with accurate correlation of the quantity of amplified catalytic subunit of telomerase with the quantity of tumor cells, are found in densities as low as 1.070 g/ml. This value of 1.070 g/ml is significantly lower than the value of 1.075 g/ml taught by Bosslet *et al.* as being the lower limit of the range of densities in which non-tumor cells are found.

The Examiner alleges that in the density range specified in the instant claims, it is the upper limit of 1.067 g/ml that is critical, and that this value is close to the upper limit of 1.068 g/ml specified in Bosslet *et al.* for the separation of tumor cells. As discussed above and below, however, and as

demonstrated by the attached DECLARATION of Dahm, both the upper and lower limits of the density range of 1.060-1.065 g/ml specified in the instant claims as amended are necessary for the accurate quantification of tumor cells from a body fluid. The lower limit avoids significant losses of tumor cells from the enriched fraction, while the upper limit avoids the presence of contaminating telomerase-positive non tumor cells in the tumor cell enriched fraction.

As discussed below, the combination of teachings of Cech *et al.*, Van Vlasselaer and Bosslet *et al.* are defective in failing to teach or suggest (i) concentration of tumor cells from a body fluid by selecting a cell separation medium density range that depletes telomerase-positive non tumor cells and avoids loss of tumor cells so that the concentrated tumor cells can be quantitated accurately; (ii) specific amplification of the catalytic subunit of telomerase from the tumor cells in a body fluid regardless of the tissue of origin of the tumor cells; (iii) the separation of tumor cells from telomerase-positive non tumor cells; and (iv) correlation of the amplified catalytic subunit of telomerase with the quantity of tumor cells in a body fluid.

The combination of teachings of the cited references does not result in the instantly claimed methods.

As discussed previously and above, the teachings of Cech *et al.* and Van Vlasselaer, singly or in combination, does not result in the instantly claimed methods because neither of the cited references, singly or in combination, teaches or suggests any method for quantifying circulating tumor cells in a body fluid. Further, neither reference, singly or in combination, teaches or suggests the concentration of tumor cells regardless of tumor cell type using a cell separation medium, nor the separation of tumor cells from telomerase-positive non-tumor cells in body fluids such as blood.

Bosslet *et al.*, directed to a density gradient centrifugation-based method for isolating tumor cells from host tissue, does not cure the deficiencies of Cech *et al.* and Van Vlasselaer. Bosslet *et al.* teaches a centrifugation-based method of separating tumor cells from their host tissue or invaded tissue, where the cell

separation medium (Percoll) is a continuous gradient from 1.051 to 1.068 g/ml. Bosslet *et al.* does not teach or suggest a method for quantitating tumor cells. There is no teaching or suggestion in Bosslet *et al.* of selecting the density range of the cell separation medium such that the enriched tumor cell fraction is (1) free of telomerase-positive non-tumor cells; and (2) avoids excessive loss of tumor cells from the enriched tumor cell fraction; so that the amount of amplified catalytic subunit of telomerase can be accurately correlated to the number of tumor cells. Since none of the cited references, singly or in any combination, teaches or suggests selection of a cell separation medium density such that tumor cells are enriched from a body fluid in a manner that allows for their accurate quantitation by (i) depleting telomerase-positive non tumor cells so that the amplified catalytic subunit if telomerase can be directly correlated to the number of tumor cells in the body fluid; and (ii) avoiding tumor cell loss so that the quantitation provides an accurate estimate of the number of tumor cells in the body fluid, the combination of the cited references cannot lead to the instant method for the quantification of tumor cells in a body fluid.

Notwithstanding the failure of the Office Action to establish a *prima facie* case of obviousness, the methods of the instant claims provides results not taught or suggested by Cech *et al.*, Van Vlasselaer or Bosslet *et al.*, singly or in any combination

Unexpected Results

Unexpected properties must always be considered when determining obviousness. It is impermissible to ignore the advantages, properties, utilities and unexpected results that flow from the claimed invention; they are part of the invention as a whole. *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983). A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesch*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

Notwithstanding the failure of the Office Action to establish a *prima facie*

case of obviousness, the methods of the instant claims provide results not taught or suggested by Cech *et al.*, Van Vlasselaer or Bosslet *et al.*, singly or in any combination. Cech *et al.* does not teach or suggest any centrifugation-based method for the enrichment of tumor cells from a body fluid; therefore, Cech *et al.* provides no teaching or suggestion regarding selection of a cell separation medium density. Van Vlasselaer, directed to the separation of tumor cells based on the density of the particular tumor cell type (breast cancer), does not teach or suggest any general method for the enrichment of tumor cells from a body fluid, much less doing so in a manner that separates tumor cells from telomerase-positive non-tumor cells while avoiding loss of tumor cells from the enriched fraction. Bosslet *et al.* teaches that tumor cells can be found in density bands ranging from 1.051 to 1.068 g/ml, whereas normal splenic leukocytes are found in bands ranging from 1.075-1.082 g/ml. As demonstrated in the executed DECLARATION under 37 C.F.R. §1.132 of Dahm, discussed below, the lower limit of the density range taught by Bosslet *et al.* for the enrichment of tumor cells, 1.051 g/ml, would lead to unacceptable losses of tumor cells that would prevent accurate quantitation of the tumor cells in a biological sample. Further, as demonstrated by the enclosed DECLARATION of Dahm, a significant amount of telomerase positive non-tumor cells that interferes with accurate correlation of the quantity of amplified catalytic subunit of telomerase with the quantity of tumor cells, is found at separation medium densities much lower than the value of 1.075 g/ml taught by Bosslet *et al.* as the lower limit of the range of densities (1.075 - 1.082 g/ml) in which non-tumor cells are found.

None of the cited references, singly or in any combination, teaches or suggests a cell separation medium density range that permits accurate quantification of tumor cells by quantitating the amplified catalytic subunit of telomerase in the enriched tumor cell fraction. On the other hand, as the accompanying DECLARATION under 37 C.F.R. § 1.132 of Michael W. Dahm demonstrates, the instantly claimed methods provide results that are not taught or suggested by any of the cited references, singly or in any combination.

As discussed below, the DECLARATION demonstrates that (i) using a cell separation medium with a density of at least about 1.060 g/ml avoids a significant loss of the tumor cells being concentrated from the body fluid; and (ii) using a cell separation medium with a density less than about 1.065 g/ml removes any contaminating telomerase-positive non-tumor cells from the concentrated tumor cell fraction. Thus, the DECLARATION demonstrates that in the instantly claimed methods for the quantification of tumor cells in a body fluid by correlating the number of tumor cells to the amount of amplified catalytic subunit of telomerase, a cell separation medium density of the range of about 1.060-1.065 g/ml is necessary for accurate quantification of the tumor cells because this range (i) depletes telomerase-positive non tumor cells from the enriched tumor cell fraction; and (ii) avoids loss of tumor cells from the enriched tumor cell fraction. None of the cited references, singly or in any combination, teaches or suggests a method that permits accurate quantification of tumor cells by selection of a cell separation medium such that telomerase-positive non tumor cells are depleted from a tumor cell fraction concentrated from a body fluid, while avoiding the loss of tumor cells from the fraction.

The DECLARATION of DAHM

An executed DECLARATION of Dahm under 37 C.F.R. §1.132 is provided herewith. The DECLARATION provides data assessing the range of cell separation medium densities required to separate tumor cells from telomerase-positive non-tumor cells using the instantly claimed methods, while avoiding the loss of tumor cells from the enriched tumor cell fraction. The data provided in the attached DECLARATION demonstrates that (i) using a density centrifugation method with a specific density of about 1.060 g/ml to 1.065 g/ml depletes telomerase positive non-tumor cells from tumor cells concentrated from a peripheral blood or urine sample while avoiding a significant loss of tumor cells; (ii) the density range from about 1.060 g/ml to 1.065 g/ml efficiently enriches and allows detection and quantification of a variety of disseminated tumor cells stemming from different solid tumors, such as breast cancer, cancers of the

gastrointestinal tract (*e.g.*, colorectal cancer), prostate cancer, lung cancer, cervical cancer and malignant melanoma; and (iii) the catalytic subunit of human telomerase (hTRT) can be employed as a tumor associated surrogate marker when tumor cells from a body fluid are enriched using a cell separation medium density ranging from about 1.060 g/ml to 1.065 g/ml.

The DECLARATION demonstrates that any density below about 1.060 g/ml will lead to unacceptable losses of tumor cells in a sample and false negative results in subsequent molecular detection reactions (*e.g.* RT-PCR) to quantitate the number of tumor cells based on the quantitation of amplified catalytic subunit of telomerase. Densities greater than about 1.065 g/ml will result in contamination of the enriched tumor cell fraction with telomerase-positive non-tumor cells, resulting in false positive results when quantitating the number of tumor cells based on the quantitation of amplified catalytic subunit of telomerase. Thus, the DECLARATION demonstrates that hTRT can be used as a specific, ubiquitous tumor-associated marker for the detection and quantification of circulating solid tumor cells in human body fluids when the tumor cells are enriched from the body fluid using a density centrifugation method in which the density of the cell separation medium ranges from about 1.060 g/ml to 1.065 g/ml. A specific density in the range of 1.060-1.065 g/ml specifically concentrates, detects and allows quantification of disseminated tumor cells in a body fluid without significant contamination from telomerase-positive non-tumor cells or significant loss of tumor cells in the sample. These results, which provide greater accuracy of the methods for quantification of tumor cells as instantly claimed, are not taught or suggested by any of the cited references, singly or in any combination.

Conclusion

1) None of the cited references nor their combination teaches or suggests a method for the quantification of tumor cells. Contrary to the Examiner's assertion that Cech *et al.* provides a method for tumor cell quantification, there is no teaching or suggestion in Cech *et al.* to obtain the

number of tumor cells in a biological sample by measuring the amount of amplified catalytic subunit of telomerase. Even if, as the Examiner alleges, correlation of the amount of amplified catalytic subunit of telomerase with the number of tumor cells was routine and well-known to one of ordinary skill in the art, there must be some teaching or suggestion in the cited reference to perform such correlation and Cech *et al.* provides no such teaching or suggestion.

2) The cited references, singly or in combination, fail to teach or suggest selection of a cell separation medium density range of 1.060-1.065 g/ml for the concentration of tumor cells from a body fluid. Cech et al. and Van Vlasselaer do not teach or suggest any density range for the concentration of tumor cells regardless of tumor cell type from a body fluid. Bosslet *et al.* teaches separation of tumor cells using linear Percoll density gradients ranging from 1.051 to 1.068 g/ml to concentrate the rumor cells, while the non-tumor cells are found at higher densities ranging from 1.075 to 1.082 g/ml. As summarized below with respect to the attached DECLARATION of Dahm, the values of the density range taught by Bosslet *et al.* for the enrichment of tumor cells that are below 1.060 g/ml (1.051-1.059 g/ml), would lead to unacceptable losses of tumor cells and the density range at which contaminating non-tumor cells may be found in the tumor cell fraction is much lower (1.065-1.070 g/ml) than the density range of 1.075-1.082 g/ml taught by Bosslet *et al.*

3) As demonstrated in the executed DECLARATION under 37 C.F.R. §1.132 of Dahm provided herein, the density range of 1.060-1.065 g/ml as instantly claimed permits quantitation of tumor cells with an accuracy that is not taught or suggested by the cited references, singly or in any combination. The DECLARATION demonstrates that in a method for the quantification of tumor cells from a body fluid by correlating amplified catalytic subunit of telomerase with the number of tumor cells as instantly claimed, any density below 1.060 g/ml would impact the accuracy of quantitation by causing significant tumor cell loss, while any density above about 1.065 g/ml would impact the accuracy of quantitation by amplifying the catalytic subunit of telomerase from the

contaminating telomerase positive non-tumor cells. The DECLARATION provides results not taught or suggested by Cech *et al.*, Van Vlasselaer or Bosslet *et al.*, singly or in any combination.

Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

B. REJECTION OF CLAIM 3 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND BOSSLET *ET AL.* AND FURTHER IN VIEW OF GWYNN *ET AL.*

Claim 3 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Van Vlasselaer and Bosslet *et al.* and further in view of Gwynn *et al.* (U.S. Patent No. 6,025,156). It is alleged that it would have been *prima facie* obvious to one of ordinary skill in the art to have modified the method of generating cDNA for the catalytic subunit of telomerase allegedly taught by Cech *et al.*, using DNAse to remove DNA from mRNA samples as allegedly taught by Gwynn *et al.*, to arrive at the subject matter of Claim 3. Reconsideration of the grounds for this rejection is respectfully requested in view of amendments herein and the following remarks.

Analysis

Claim 3 is directed to the method of Claim 2 where the sample is treated with a DNAase prior to preparing cDNA. As discussed above, neither Cech *et al.*, Van Vlasselaer, or Bosslet *et al.*, singly or in combination, teaches or suggests any method for the quantification of circulating tumor cells in a body fluid, much less a step of correlating expression of the catalytic subunit of telomerase from the tumor cells with the number of tumor cells. Further, as also discussed above and as demonstrated by the attached DECLARATION of Dr. Dahm, none of the cited references, singly or in any combination, teaches or suggests a cell separation medium density range (1.060-1.065 g/ml) to effect tumor cell enrichment in a manner that depletes telomerase-positive non tumor cells from the tumor cell fraction, while avoiding loss of tumor cells. Gwynn *et al.*, directed to Topoisomerase III, does not cure these deficiencies. Therefore, a

combination of the cited references cannot lead to the subjected matter of rejected Claim 3. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

C. REJECTION OF CLAIM 29 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND BOSSLET *ET AL.* AND FURTHER IN VIEW OF SELBY

Claim 29 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Van Vlasselaer and Bosslet *et al.* and further in view of Selby (GB Patent No. 2 260 811). It is alleged that it would have been *prima facie* obvious to one of ordinary skill in the art to combine the method of centrifugation allegedly taught by the combination of Cech *et al.*, Van Vlasselaer and Bosslet *et al.*, with Selby, which allegedly teaches cooling the sample after centrifugation, an element of Claim 29, was routinely practiced in the art. Reconsideration of the grounds for this rejection is respectfully requested in view of amendments herein and the following remarks.

Analysis

Claim 29 specifies that in the method of Claim 1, after centrifugation and before collecting the tumor cell-enriched interface, the centrifugation vessel is removed and cooled to prevent mixing of the cells in the different layers.

As discussed above and as demonstrated by the attached DECLARATION of Dr. Dahm, neither Cech *et al.*, Van Vlasselaer, nor Bosslet *et al.*, singly or in combination, teaches or suggests a method for quantifying circulating tumor cells in a body fluid that are concentrated from the body fluid by treatment with a cell separation medium with density in the range of about 1.060-1.065 g/ml that separates tumor cells from telomerase positive non-tumor cells while avoiding significant loss of tumor cells from the concentrated tumor cell fraction. Cech *et al.*, Van Vlasselaer or Bosslet *et al.*, singly or in any combination, also do not teach or suggest any step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in a body fluid. Selby,

directed to diagnosis or monitoring of cancer using coamplification to detect and quantify tissue-specific genes, does not cure these deficiencies.

The combination of the cited references, each of which lack the elements of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells after concentrating tumor cells from a body fluid using a cell separation medium density in the range of 1.060-1.065 g/ml, does not result in the subject matter of rejected Claim 29. Therefore, the Examiner has failed to set forth a case of *prima facie* obviousness.

D. REJECTION OF CLAIMS 5 AND 6 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND BOSSLET *ET AL.* AND FURTHER IN VIEW OF GELMINI *ET AL.*

Claims 5 and 6 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Van Vlasselaer and Bosslet *et al.* and further in view of Gelmini *et al.* (*Clin. Chem. 43(5)*: 752-758 (1997)). It is alleged that it would have been *prima facie* obvious to one of ordinary skill in the art to combine the amplification method of Cech *et al.* with the method of Gelmini *et al.*, which allegedly teaches real time quantitative PCR. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

Dependent Claim 5 specifies that in the method of Claim 1 for the quantification of tumor cells in a body fluid, the amplification products of the catalytic subunit of telomerase are labeled during amplification and the amplification kinetics are measured continuously, including during the amplification process. Claim 6 further specifies that a probe that is specific for the amplification products and that emits a characteristic signal proportional to the products amplified per synthesis cycle, is present during amplification.

As discussed above, none of Cech *et al.*, Van Vlasselaer and Bosslet *et al.*, singly or in any combination, teaches or suggests a method for quantifying circulating tumor cells regardless of tumor cell type in a body fluid by

concentrating the tumor cells with a cell separation medium density in the range of 1.060-1.065 g/ml that separates tumor cells from telomerase positive non-tumor cells while avoiding tumor cell loss.

Gelmini *et al.*, directed to quantitative PCR of the c-*erb*B-2 oncogene, does not cure these deficiencies. The combination of the cited references does not lead to the principal elements of Claims 5 and 6. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

E. REJECTION OF CLAIMS 13 AND 74 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND BOSSLET *ET AL.*

Claims 13 and 74 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Van Vlasselaer and Bosslet *et al.* It is alleged that it would have been *prima facie* obvious to one of ordinary skill in the art to select primers to amplify all or part of the catalytic subunit of telomerase gene using the parameters according to "routine methods" allegedly taught by Cech *et al.* Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks. It is respectfully submitted that this rejection has been rendered moot with respect to Claim 74, which is cancelled herein.

Analysis

Claim 13 specifies that in the method of Claim 1 for the quantification of tumor cells in a body fluid, the amplification primers whose sequences are set forth in SEQ ID. NOS. 1 and 2 are used for the amplification of the catalytic subunit of telomerase mRNA.

As discussed above, neither Cech *et al.*, Van Vlasselaer, nor Bosslet *et al.*, singly or in any combination, teaches or suggests a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment regardless of tumor cell type with a cell separation medium in the density range of 1.060-1.065 g/ml for separating tumor cells from telomerase

positive non-tumor cells while avoiding significant loss of tumor cells from the concentrated fraction.

It is alleged that the primers whose sequences are set forth in SEQ ID. NOS. 1 and 2 and that are elements of Claim 13 were merely selected by the "routine methods" provided by Cech *et al.* for the amplification of all or part of the catalytic subunit of telomerase. As discussed above, however, and as demonstrated by the attached DECLARATION of Dahm, the cited references do not teach or suggest principal elements of Claim 13, such as a method for quantifying circulating tumor cells in a body fluid that includes a step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells; or adjusting the cell separation medium to a density range that effects separation of tumor cells from telomerase positive non-tumor cells while minimizing tumor cell loss. Therefore, even if, as the Examiner alleges, Cech *et al.* provides primers for the quantitative amplification of the catalytic subunit of telomerase and parameters for their selection, Cech *et al.*, singly or in combination with the remaining cited references, does not provide any of the principal elements of Claim 13 as set forth above. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

F. REJECTION OF CLAIMS 12 AND 57-59 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND BOSSLET *ET AL.* AND FURTHER IN VIEW OF MELVIN *ET AL.*

Claims 12 and 57-59 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Van Vlasselaer and Bosslet *et al.* and further in view of Melvin *et al.* (WO 97/12246). It is alleged that it would have been *prima facie* obvious to one of ordinary skill in the art to have modified the method of Cech *et al.* to include controls such as β-actin as a positive control and sterile water as a negative control as allegedly taught by Melvin *et al.*, to arrive at the claimed subject matter. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

Claim 12 is directed to the method of Claim 1 in which water is employed as a negative control. Claim 57 is directed to the method of Claim 11, which specifies that the sample used in the method of Claim 1 is peripheral blood. Claim 57 specifies that as a positive control, a nucleic acid that occurs in peripheral blood is specifically amplified and detected. Claim 58 further specifies positive controls such as β-globin, glyceraldehyde phosphate dehydrogenase, β-actin or a T-cell receptor. Claim 59 is directed to the method of Claim 3 where no reverse transcription reaction is carried out before amplification and/or water is used in the amplification as a negative control. All of the claims either directly or indirectly depend from Claim 1.

As discussed above, neither Cech *et al.*, Van Vlasselaer nor Bosslet *et al.*, singly or in any combination, teaches or suggests a method for quantifying circulating tumor cells in a body fluid that are concentrated from a body fluid by treatment with a cell separation medium of density in the range of 1.060-1.065 g/ml for separating tumor cells from telomerase positive non-tumor cells while avoiding significant loss of tumor cells. The cited references also do not teach or suggest any step of correlating the amount of amplified catalytic subunit of telomerase with the number of tumor cells in a body fluid.

Melvin *et al.*, directed to the detection of CYP1B1 in cancer cells, does not cure these deficiencies. The combination of the cited references does not result in the principal elements of rejected Claims 12 and 57-59. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

G. REJECTION OF CLAIMS 30-33, 65 AND 66 UNDER 35 U.S.C. § 103(a) OVER CECH *ET AL.* IN VIEW OF VAN VLASSELAER AND BOSSLET *ET AL.* AND FURTHER IN VIEW OF OKA *ET AL.*

Claims 30-33, 65 and 66 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the teachings of Cech *et al.* in view of Van Vlasselaer and Bosslet *et al.* and further in view of Oka *et al.* (U.S. Patent No. 5,298,165). It is alleged that it would have been *prima facie* obvious to one of

ordinary skill in the art to have modified the method of Cech *et al.* for the quantitation of tumor cells, in view of Van Vlasselaer and Bosslet *et al.*, with different membranes, filters or porous barriers allegedly taught by Oka *et al.*, to arrive at the subject matter of the rejected claims. It is further alleged that the pore size and thickness of filters are "routinely optimizable" based upon the desired parameters, since Oka *et al.* allegedly teaches how densities may be determined. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Analysis

Claim 30 is directed to the method of Claim 1 in which the centrifugation vessel is divided into two compartments by a porous barrier, filter or sieve, and the body fluid is introduced into the upper compartment. Claims 31, 32, 65 and 66 specify the pore size of the porous barrier, filter or sieve, and Claim 33 specifies that at least one of the porous barrier, filter or sieve is fabricated from or coated with a hydrophobic material. All of the rejected claims are either directly or indirectly dependent on Claim 1.

As discussed above, neither Cech *et al.*, Van Vlasselaer nor Bosslet *et al.*, singly or in any combination, teaches or suggests a method for the quantification of tumor cells in a body fluid where the tumor cells are concentrated from the body fluid by treatment with a cell separation medium of density 1.060-1.065 g/ml, nor of correlating the amount of amplified mRNA encoding the catalytic subunit of telomerase with the number of tumor cells in a body fluid. Oka *et al.*, directed to removal of leukocytes from leukocyte-containing blood products to reduce the side effects of blood transfusions, fails to cure these deficiencies. While Oka *et al.* may provide for the use of filters in its methods, Oka *et al.* does not teach or suggest concentration of tumor cells in a body fluid, much less by treatment and/or centrifugation with a cell separation medium of density in the range of 1.060-1.065 g/ml. Hence, Oka *et al.* does not cure the principal deficiencies in the teachings of Cech *et al.*, Van Vlasselaer and Bosslet *et al.*

The combinations of teachings fail to suggest several elements of the claimed methods as discussed above. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

* * *

In view of the remarks herein, examination of the application on the merits and allowance is respectfully requested.

Respectfully submitted,
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